bio-protocol Preprint

Not Peer Reviewed
Ask a question online

DUST assay in human cells

Yilun Sun John L. Nitiss Yves Pommier

Updated date: Oct 8, 2021

An abbreviated version of this protocol was published in Science Advances in Nov 2020

A conserved SUMO pathway repairs topoisomerase DNA-protein cross-links by engaging ubiquitin-mediated proteasomal degradation

DOI: 10.1126/sciadv.aba6290

Detailed protocol

1. Seed 5 × 10^5 HEK293 cells in 35-mm dish per sample the day before treatment.
2. Next day, add topoisomerase inhibitors of desired concentrations to the cells.
3. Collect cells after treatment by washing with 1× PBS then lysing with 600 µl of DNAzol (Invitrogen) containing 1 × protease cocktail inhibitor and 1 mM DTT for 10 min on ice.
4. Add 300 µl 200 proof ethanol to the dishes and place the dishes on a shaker at 4 °C till white aggregates (dehydrated DNA) become visible.
5. Transfer the lysates to 1.5 mL eppendorf tube for centrifuge at 15,000 rpm at 4 °C.
6. Remove the supernatant and wash the nucleic acid pellet at the bottom of the tube with 75% ethanol. Remove the ethanol and resuspend the pellet in 200 µl of TE buffer,
7. Heat the nucleic acid sample at 37°C for 15 min, followed by shearing with sonication (40% output for 10-s pulse and 10-s rest for four times).
8. Centrifuge the samples at 15,000 rpm for 5 min at 4°C, collect the supernatant and treat with RNase A (100 µg/ml) for 1 hour at 37°C to remove RNA contamination (**the *RNA removal step is optional if the yield of nucleic acid is low***), followed by addition of 1:10 volume of 3 M sodium acetate sodium acetate and 2.5 volume of 200 proof ethanol.
9. After 20 min 15,000 rpm centrifugation, recover and resuspend the DNA pellet in 100 µl of ddH_2O.
10. Take 1 µl per sample for spectrophotometric measurement of absorbance at 260 nm to quantitate DNA content (NanoDrop).
11. DNA (10 µg) from each sample was digested with 50 units of micrococcal nuclease (100 units/µl; Thermo Fisher Scientific) in the presence of 5 mM $CaCl_2$ at 37°C for 30 min.
12. Add 4 × SDS sample loading buffer to the digested samples, followed by gel electrophoresis on 4 to 15% precast polyacrylamide gel (Bio-Rad) for immunodetection for total TOP-DPCs (Anti-TOP1, mouse monoclonal BD Biosciences Cat# 556597, 1:500; Anti-TOP2α, mouse monoclonal Millipore Cat# MAB4197, 1:500; Anti-TOP2β, mouse monoclonal BD Biosciences Cat# 611492, 1:500), SUMO-TOP-DPCs (Anti-SUMO-1, rabbit monoclonal Cell Signaling Cat# 4940, 1:250; Anti-SUMO-2/3, rabbit monoclonal Cell Signaling Cat# 4971, 1:250, and Ub-TOP1-DPCs (Anti-ubiquitin, mouse monoclonal Santa Cruz Cat# sc-8017, 1:100;)
13. 2 µg of each sample (without micrococcal nuclease digestion) was subjected to slot-blot for immunoblotting with anti–double-stranded DNA antibody (Anti-dsDNA, mouse monoclonal Abcam Cat# ab27156, 1:5000) as a loading control to verify that amounts of DNA were digested with micrococcal nuclease.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Sun, Y. , Nitiss, J. and Pommier, Y. (2021). DUST assay in human cells. Bio-protocol Preprint. bio-protocol.org/prep1399.
2. Sun, Y., Jenkins, L. M. M., Su, Y. P., Nitiss, K. C., Nitiss, J. L. and Pommier, Y.(2020). A conserved SUMO pathway repairs topoisomerase DNA-protein cross-links by engaging ubiquitin-mediated proteasomal degradation . Science Advances 6(46). DOI: 10.1126/sciadv.aba6290